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Variation in the Phytochemical Constituents of Seeds, Mature and Immature Leaves of Moringa oleifera Lam. Growing in Five Local Government Areas of Oyo State, Nigeria

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Abstract
The study was conducted to determine the variation in the quantities of phytochemicals present in *Moringa oleifera* Lam. seeds and leaves in five Local Government Areas of Oyo State, Nigeria. The study area covered two villages from each local government area where leaves and seeds of *M. oleifera* were collected, washed, air-dried and ground to powdery form. Phytochemical analysis carried out on the extract showed that the plant samples contained flavonoids, tannins, alkaloids and saponins which could be attributed to the medicinal usefulness of the plant for treating ailments such as diarrhea, menstrual pain, fever, malaria and stomach disorder. The quantitative phytochemical analysis of *M. oleifera* showed that the seeds had the highest percentages in alkaloids, flavonoids, tannins and saponins when compared with the leaves, with Saki-West samples having the highest value. The mature leaves had higher quantities of the phytochemicals in all the samples compared with the immature leaves with samples of mature leaves from Saki West Local Government also having the highest value when compared with samples from other areas of study. Based on the findings, the seeds and the mature leaves of *Moringa oleifera* should be preferentially used for medicinal purpose.
Keywords: Phytochemical constituents, leaves (mature and immature), seeds, *Moringa oleifera*

1. Introduction

Plants are the richest source of drugs for traditional medicine, modern medicines, food supplements, folk medicine, pharmaceutical intermediates and chemical entities for synthetic drugs (Mabberly, 1997). Isolated active principles have provided leads in the development of several lifesaving drugs, which are in use today and are generally called phytochemicals (Anwar and Rashid, 2007). These phytochemicals play protective roles in plants, each chemical works in different ways, not all are the same for human and not all come from the same plant. Some have shown more promises than others in fighting diseases in humans. Elujoba *et al.,* (2009) reported that the use of traditional medicine in the treatment and management of an array of disease in the African continent is likely to continue due to Africa's sociocultural, socioeconomic heritage, lack of basic healthcare and support for the rural population.

Moringa oleifera Lam. which belongs to the family Moringaceae is commonly known as drumstick or horseradish and is native to Sub Himalaya tracts of India, Pakistan, Bangladesh, Central America Afghanistan and Africa (Fahey, 2005). *M. oleifera,* a highly nutritional plant is used in Asia as vegetable and medicinal plant; this is attributed to the presence of proteins, vitamins and various phenol compounds in the oil (Anwar *et al.,* 2007). The diverse range of medicinal uses for *Moringa oliefera* include its use as antioxidant, antibacterial, anti-inflammatory, anti-fungal, antispasmodic and its diuretic properties as well as its wound healing ability. Additionally, the root bark has been used as an analgesic and treatments for heart complaints, as well as for eye diseases, inflammation and dyspepsia (Caceres *et al.,* 1991). A report by Ndiaye *et al.,* (2002) stated that *Moringa oleifera* is used in Africa folk medicine to treat rheumatic and articular pains.

Over the past two decades, many reports have appeared in the mainstream scientific journals describing its nutritional and medicinal properties. *Moringa oleifera* is a widely cultivated species and has now naturalized in West Africa and Nigeria in particular (Bharali *et al.,* 2003). Although *M. oleifera* is claimed to have a lot of economic values such as medicinal and nutritional values, it has been observed that most people who use the plant for its medicinal properties do not really know the parts of the plant that contain the highest percentage of the phytochemical constituents. As reported by Singh *et al.,* (2010), the variation in the quantities of phytochemical constituents in plant and its parts is greatly influenced by environmental condition and degree of development. It has also been reported that some plants reveal the presence of bioactive properties in different parts (leaves, stem bark, seeds and roots) in various degrees or concentration which also reflects in their therapeutic efficacy (Musyimi *et al.,* 2005; Akharaiyi and Boboye, 2010).

This study was carried out to ascertain the variation of phytochemicals in mature and immature leaves as well as the seeds of *Moringa oleifera* growing in five local government areas of Oyo state. This is to advance the knowledge of potential users of the plant parts in phytomedicinal preparations.

2. Materials and Method

2.1 Collection of plant materials

Five Local Government Areas of Oyo state; Iddo, Saki West, Ibarapa East, Ogbomoso South and Afijio were visited for collection of plant materials. *Moringa oleifera* seeds as well as the mature and immature leaves (Plates 1 & 2) were collected from two villages in each of the local government areas.

The plant materials were air-dried indoor for a week and further dried in the oven at 30°C. Thereafter, the dried samples were ground into powder using an electric blender and stored in air-tight glass bottles at 4°C prior to analysis.

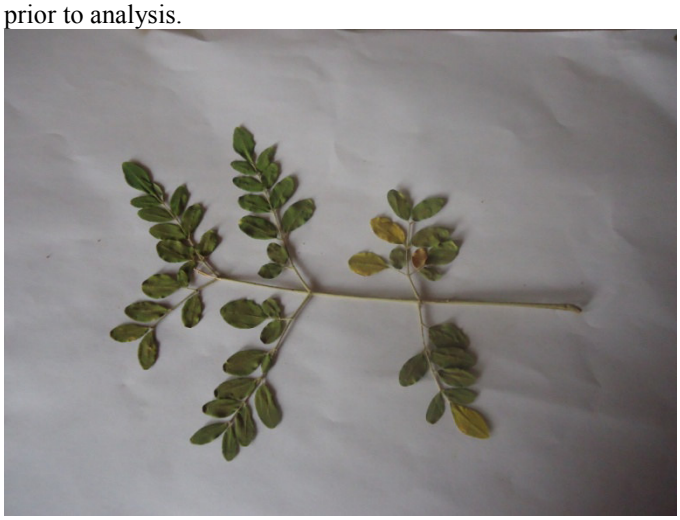

Plate 1: Mature leaves of *M. oleifera*

Plate 2: Immature leaves of *M. oleifera*

2.2 Quantitative analysis of plant parts

2.2.1 Determination of alkaloids

Two grams of finely grinded sample was weighed into a 100ml beaker and 20mls of 80% absolute alcohol added to give a smooth paste. The mixture was transferred to a 250ml flask and more alcohol was added to make up 100ml and 1g of magnesium oxide was added. The mixture was digested in a boiling water bath for 1.5 hours under a reflux condenser with occasional shaking (Allen, 1992). The residue was returned to the flask and redigested for 30minutes with 50ml alcohol after which the alcohol was evaporated, adding hot water to replace the alcohol lost. When all the alcohol had been removed, 3 drops of 10% HCl was added.

The whole solution was later transferred into a 250ml volumetric flask, 5ml solution of Zinc acetate and 5ml of Pottasium ferrocyanide was added. The flask was allowed to stand for a few minutes, filtered through a dry filter paper and 100ml of the filtrate was transferred into a separatory funnel and the alkaloids present were extracted vigorously by shaking with 5 successive portions of chloroform (Henry, 1993). The residue obtained was dissolved in 10ml hot distilled water and transferred into a kjeldahl tube with the addition of 0.20g sucrose and 10ml conc. $H_2S0_4$and 0.02g selenium for digestion to a colorless solution to determine %N by kjeldahl distillation method. % Nitrogen got is converted to % Total alkaloid= %NX3.26, % alkaloid =% NX3.26

2.2.2 Determination of flavonoids

0.5g of finely grinded sample was weighed into a 100ml beaker and 80ml of 95% Ethanol added and stirred with a glass rod to prevent lumping (Allen, 1979). The mixture was filtered through a Whatman No. 1 filter into a 100ml volumetric flask and made up to mark with ethanol. 1ml of the extract was pipetted into 50ml volumetric flask, four drops of Conc. HCl was added via a dropping pipette after which 0.5g of magnesium turnings added to develop a magenta red coloration. Standard flavonoid solutions 0-5ppm were prepared from 100ppm stock solution and treated in a similar way with HCl and magnesium turnings like the sample and standard solutions were read on a digital Jenway V6300 spectrophotometer at a wavelength of 520nm.

The percentage flavonoid was calculated using this formula:

$$\frac{\text{Absorbance of sample X Average gradient factor X gradient factor X Dilution factor}}{\text{Weight Sample X 10,000}}$$

2.2.3 Determination of tannins

0.20g of sample was measured into a 50ml beaker, 20ml of 50% methanol was added and covered with paraffin and placed in a water bath at 77-90^{o}C for 1 hour. It was shaken thoroughly to ensure a uniform mixing. The extract was quantitatively filtered using a double layered Whatman No. 1 filter paper into a 100ml volumetric flask; 20ml of water, 2.5ml Folins-denis reagent and 10ml of 11% Na_2C0_3 were added and mixed properly. The mixture was made up to mark with water mixed well and was allowed to stand for 20minutes (Swain, 1979). The bluish green colour was developed at the end of the range. The standard solutions, 0-10ppm were treated similarly as 1ml sample above. The absorbance of the Tannic acid standard solutions as well as samples was read after colour development on a spechronic21D spectrophotometer at a wavelength of 760nm.

% Tannin was calculated using the formula:

$$\%\ \text{Tannin} = \frac{\text{Absorbance of sample X Average gradient factor X Dilution factor}}{\text{Weight of sample X 10,000}}$$

2.2.4 Determination of saponins

The spectrophotometric method of Brunner (1984) was used for saponin analysis. 1g of finely grinded sample was weighed into a 25ml beaker and 100ml of Isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter the mixture was filtered through a Whatman No. 1 filter paper into a 100ml beaker and 20ml of 40% saturated solution of Magnesium carbonate ($MgCO_3$) was added. The mixture obtained with $MgC0_3$was again filtered through a Whatman No. 1 filter paper to obtain a clear colourless solution. 1ml of the colourless solution was pipetted into 50ml volumetric flask and 2ml of 5% $FeCl_3$ solution was added and made to mark with distilled water (Brunner, 1984). It was allowed to stand for 30 minutes for blood red colour to develop. 0-10ppm standard saponin was prepared for saponin stock solution. The absorbance of the samples as well as standard saponin solution was read after colour development in a Jenway V6300 Spectrophotometer at a wavelength of 380nm.

$$\%\ \text{Saponins} = \frac{\text{Absorbance of samples X Gradient factor X Dilution factor}}{\text{Weight of sample X 10,000}}$$

3. Results and Discussion

Table 4.1 shows the percentage composition of alkaloids, flavonoids, tannins and saponins present in all the samples of *Moringa oleifera* collected from the five different Local Governments areas (LGAs) of Oyo State.

This study has shown that the seeds have the highest percentage composition of the bioactive constituents in all the samples from the different locations. This may be attributed to the fact that the seeds are regarded as storage organs of different bioactive compounds and that in most cases, maximum accumulation of chemical constituents occur at the time of flowering and subsequent formation of seeds which then decline at the beginning of the fruiting stage (Mendonca-Filho, 2006). This was also observed by Makinde *et al* (1994) in their report on *Morinda lucida* extract against *Plasmodium berghei* in mice.

In all the samples collected from the different locations, the mature leaves contained the highest composition of all the bioactive constituents analysed. This agrees with the report of Sreelatha and Padma, (2009) who observed that *M. oleifera* leaf extract contains polyphenols, therefore the antioxidant effects of the leaf extract may depend on its phenol components. So also, it was gathered by the same workers that the non-enzymatic antioxidants increase with maturity in *M. oleifera* leaves hence deduced that the best stage of *M. oleifera* leaves suited for consumption is the mature stage, when the maximum benefit of the antioxidant content can be derived. This same observation agrees with the report given by Singh *et al.,* (2010) on variation of some phytochemicals in methi and saunf plants at different stages of development. From the report, the workers observed that phytochemicals showed significant increase in mature plants in comparison to early stage plant and their parts in both methi and saunf therefore suggesting that these phytochemicals increase with maturity. This may indicate that at a certain period of the year, the bioactive components present in some plant specimen may

have reduced to minimum levels such that when these plants are tested again, contrary results may be obtained.

This could explain why certain plants are used within certain periods of the year for effective cure of diseases in ethnomedicine (Adomi and Umukoro, 2010). This observation is however contrary to the views of some *M. oleifera* users as regards the effectiveness of both the immature and mature leaves. Such people believed that the immature leaves were more effective when compared to the mature leaves and this could be attributed to the belief of the people that the immature leaves are easier to chew and tasty thereby believing it to be the more effective thus passing the information round the neighbourhood. On the contrary, phytochemical analysis of the plant sample has revealed that the phytochemicals increase with maturity which totally disagreed with the people's opinion, hence the seeds and mature leaves have more medicinal values.

4. Conclusion

Moringa oleifera has the highest percentage composition in alkaloids and flavonoids but low in its tannins and saponin composition. The presence of these phytochemicals in the plant sample contributed to their antimicrobial, antifungal, antibacterial, diuretic and analgesic properties.

The use of *M. oleifera* by the entire populace must be encouraged in order to maximize the healing of various diseases and ailments without depending on orthodox medicine. *M. oleifera* should therefore be cultivated by herb sellers, herbal medical practitioners and individuals as household herbs. However, the seeds and the mature leaves of *M. oleifera* having the highest composition of the phytochemicals are recommended for effective use of its medicinal properties.

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Table 4.1: Quantitative analysis of the bioactive constituents of *Moringa oleifera*

Sample Description	Alkaloid %	Flavonoid %	Tannin %	Saponnin %
Ibarapa East Mature leaves	0.034	0.0047	0.0011	0.017
Immature leaves	0.026	0.0039	0.0005	0.014
Seeds	0.059	0.0083	0.038	0.063
Ogbomoso Mature Leaves	0.039	0.0054	0.0016	0.024
Immature Leaves	0.028	0.0043	0.009	0.016
Seeds	0.073	0.0091	0.044	0.069
Saki West Mature Leaves	0.043	0.0056	0.0021	0.031
Immature Leaves	0.031	0.0045	0.0011	0.022
Seeds	0.085	0.0097	0.052	0.075
Iddo Mature Leaves	0.031	0.0042	0.0018	0.0026
Immature Leaves	0.022	0.0034	0.0013	0.019
Seeds	0.048	0.0071	0.041	0.066
Afijio Mature Leaves	0.036	0.0038	0.0014	0.023
Immature Leaves	0.024	0.0027	0.001	0.017
Seeds	0.055	0.0065	0.037	0.059

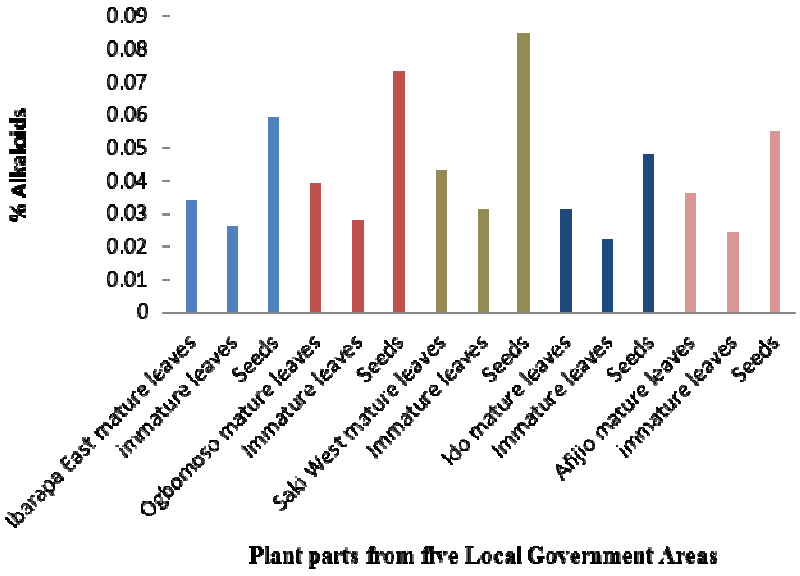

Fig 1: Percentage quantity of Alkaloids in *M. oleifera* plant parts in five LGAs of Oyo state.

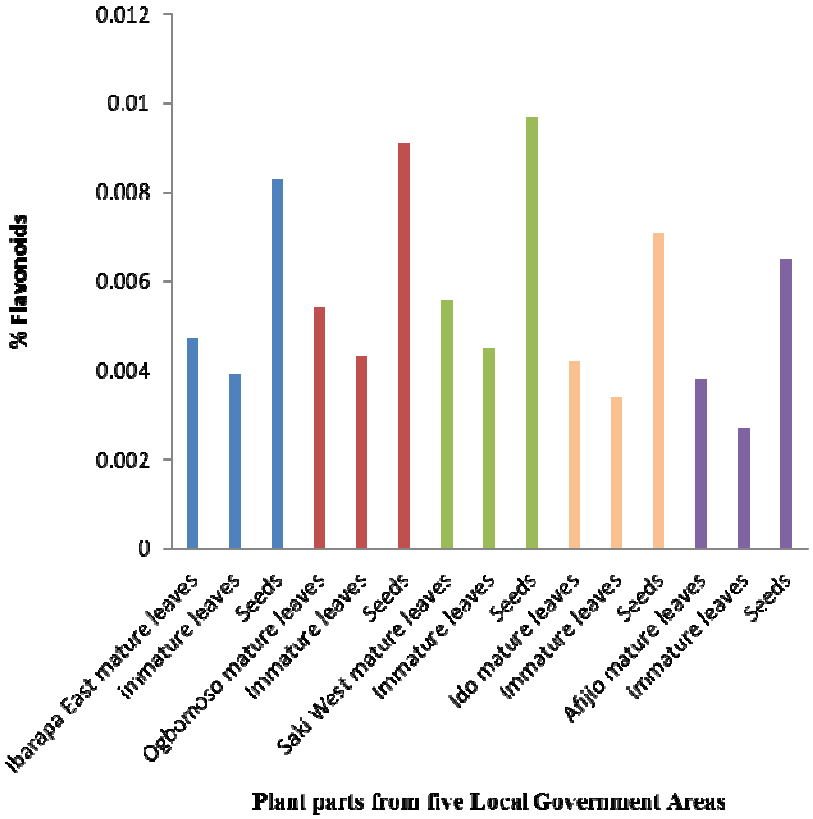

Fig 2: Percentage quantity of Flavonoids in *M. oleifera* plant parts in five LGAs of Oyo state.

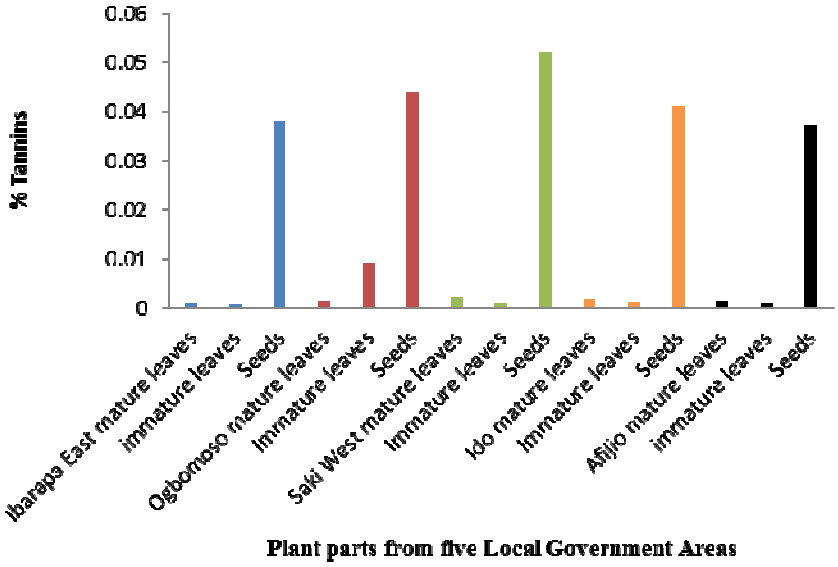

Fig 3: Percentage quantity of Tannins in *M. oleifera* plant parts in five LGAs of Oyo state.

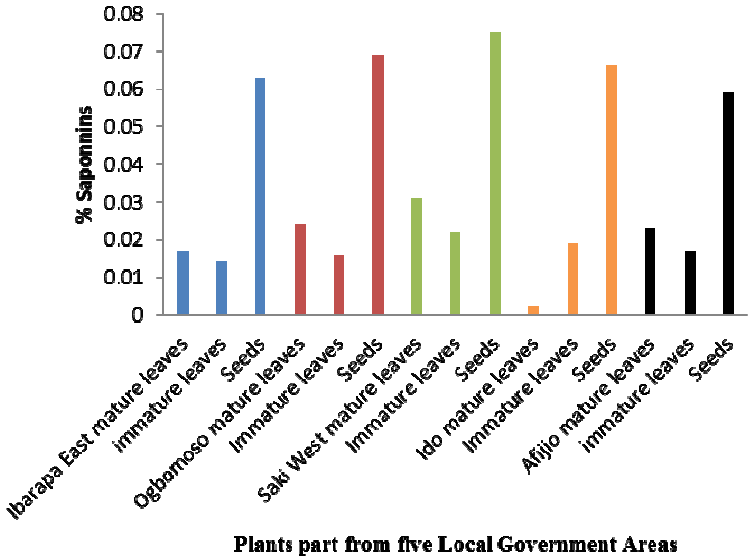

Fig 4: Percentage quantity of Tannins in *M. oleifera* plant parts in five LGAs of Oyo state.